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(54) Title: METHODS AND REAGENTS FOR MOLECULAR CLONING

(57) Abstract: The present invention provides compositions, methods, and kits for covalently linking nucleic acid molecules. The methods include a strand invasion step, and the compositions and kits are useful for performing such methods. For example, a method of covalently linking double stranded (ds) nucleic acid molecules can include contacting a first ds nucleic acid molecule, which has a topoisomerase linked to a 3' terminus of one end and has a single stranded 5' overhang at the same end, with a second ds nucleic acid molecule having a blunt end, such that the 5' overhang can hybridize to a complementary sequence of the blunt end of the second nucleic acid molecule, and the topoisomerase can covalently link the ds nucleic acid molecules. The methods are simpler and more efficient than previous methods for covalently linking nucleic acid sequences, and the compositions and kits facilitate practicing the methods, including methods of directionally linking two or more ds nucleic acid molecules.



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# INTERNATIONAL SEARCH REPORT

International Application No

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## A. CLASSIFICATION OF SUBJECT MATTER

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A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHENG CHONGHUI ET AL: "Recombinogenic flap ligation pathway for intrinsic repair of topoisomerase IB-induced double-strand breaks." MOLECULAR AND CELLULAR BIOLOGY, vol. 20, no. 21, November 2000 (2000-11), pages 8059-8068, XP002205828 ISSN: 0270-7306 cited in the application the whole document --- -/--	21-61

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

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\*O\* document referring to an oral disclosure, use, exhibition or other means

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\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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## INTERNATIONAL SEARCH REPORT

Int. Patent Application No.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHENG CHONGHUI ET AL: "DNA strand transfer catalyzed by vaccinia topoisomerase: Ligation of DNAs containing a 3' mononucleotide overhang." NUCLEIC ACIDS RESEARCH, vol. 28, no. 9, 1 May 2000 (2000-05-01), pages 1893-1898, XP002205829 ISSN: 0305-1048 the whole document ---	33
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In<sup>t</sup>ernational Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SHUMAN S: "RECOMBINATION MEDIATED BY VACCINIA VIRUS DNA TOPOISOMERASE I IN ESCHERICHIA COLI IS SEQUENCE SPECIFIC" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 88, November 1991 (1991-11), pages 10104-10108, XP002925317 ISSN: 0027-8424 cited in the application the whole document</p>	
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International Application No

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